

Biogeographic history of the endangered dwarf buffalo, subgenus *Anoa* (Bovidae: *Bubalus quarlesi* and *Bubalus depressicornis*): a perspective based on mitochondrial DNA phylogeny

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Abstract

The *Anoa*, including the Mountain *Anoa* (*Bubalus quarlesi*) and the Lowland *Anoa* (*Bubalus depressicornis*) according to current taxonomy, are endangered bovid species endemic to the tropical island of Sulawesi. They are grouped in their own subgenus *Anoa*. The historical biogeography and phylogeny of this subgenus have not been well characterized. There are two hypotheses describing the colonization routes of large mammals in Southeast Asia: land-bridges and insular route. The present study aimed to understand the molecular phylogeny and test the historical colonization route of *Anoa*. A total of 71 mitochondrial DNA (cyt b gene and control region) sequence datasets from *Anoa* and other related species were analyzed. Molecular phylogeny reconstruction was done using a Bayesian inference model. Calibration points were used to estimate divergence time in *Anoa* based on a Bayesian phylogeny tree. Estimation of ancestral areas in *Anoa* ancestors using Reconstruct Ancestral State in Phylogenies was carried out to test the *Anoa* colonization route. The phylogenetic tree revealed the existence of two groups of *Anoa*, lowland and mountain *Anoa*, which diverged during the Middle Pleistocene 1.42 Mya (highest posterior density interval: 1.14–1.71 Mya). The Sundaland area is the most probable ($P > 0.7$) ancestral area for the *Bubalus* spp. in the Indonesian archipelago. The most probable *Anoa* colonization route appears to be through land-bridges, which geological records indicate formed between Sundaland and Sulawesi during the Pleistocene.

Key Words

Anoa, historical biogeography, insular dwarfism, Land-bridge hypothesis, mtDNA phylogeny

Introduction

The genus *Bubalus* includes several species of wild and domesticated buffaloes, primarily found in Asia and Africa. The most notable species within this genus are the water buffalo (*Bubalus bubalis*), the *Anoas* (*Bubalus depressicornis* and *Bubalus quarlesi*), and the African buffalo (*Syncerus caffer*), which is sometimes classified under the same genus due to historical taxonomic confusion (Bibi and Vrba 2010). The *Anoas*, specifically, are endemic to In-

donesia and are recognized for their smaller size compared to their relatives, which is a result of insular dwarfism, a phenomenon where species evolve to be smaller in size on islands due to limited resources and space (Benítez-López et al. 2020). The taxonomy of *Bubalus* has undergone revisions, with ongoing debates regarding the classification and relationships among species, particularly between domesticated and wild forms (Bibi and Vrba 2010).

The *Anoa*, comprising *Bubalus quarlesi* and *Bubalus depressicornis* in the subgenus *Anoa*, have declined by

90% over the past 16 years, and they are even believed to be extinct in some locations. In 2016, the International Union for the Conservation of Nature (IUCN) Red List categorized both the Mountain *Anoa* (*Bubalus quarlesi*) and the Lowland *Anoa* (*Bubalus depressicornis*) as endangered species, with estimated populations of fewer than 2,500 individuals for each species (Burton et al. 2005). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) included it in Appendix I in 2010, which means that *Anoa* are protected and cannot be traded. *Anoa* are grouped into lowland and mountain *Anoa* based on morphology and habitat. The taxonomic ambiguity surrounding the subgenus *Anoa*, is a significant issue in the field of wildlife conservation and biology (Burton et al. 2005). This ongoing debate highlights the necessity for comprehensive genetic and morphological assessments to clarify the taxonomic status of these endemic species and ensure effective conservation strategies (Tanaka et al. 1996; Pitra et al. 1997; Priyono et al. 2018, 2020, 2022).

Bubalus, a genus of Asiatic bovines within the family Bovidae, includes species that vary in body size. In descending order, from largest to smallest, these species are: *Bubalus arnee* (Kerr 1792) also known as the wild water buffalo, and its domesticated form, *Bubalus bubalis* (Linnaeus, 1758), from mainland Asia; tamaraw (*Bubalus mindorensis* Heude, 1888) from Mindoro Island, Philippines; *Bubalus cebuensis* (Croft, Heaney, Flynn & Bautista, 2006; extinct) from Cebu Island; *Bubalus depressicornis* (Smith, 1872), known as the lowland *Anoa*, from lowland areas of Sulawesi Island; and *Bubalus quarlesi* (Ouwens, 1910), known as the mountain *Anoa* (Fig. 1). Average body weight for each species, are provided in Fig. 1 to facilitate comparison, based on previous studies (Rozzi 2017, 2018; Shrestha et al. 2019). This size reduction is often attributed to limited resources, reduced predation pressures, and ecological niches that favor smaller body sizes (Benítez-López et al. 2021). For example, studies have shown that large mammals, such as elephants and hippos, significantly reduce size when isolated on islands (Benítez-López et al. 2021). Other mammals in Sulawesi, such as *Stegodon sompoensis* (Falconer, 1847) and *Elephas celebensis* (Hooijer, 1949), have also evolved according to insular dwarfism (Hooijer 1949). In the case of the *Anoas*, their smaller stature compared to other buffalo species is a direct result of these insular pressures, allowing them to thrive in the unique ecological conditions of Sulawesi (Benítez-López et al. 2021). Tougaard (2001) stated that body size gradation, especially in large mammals, correlated with the possibility of their colonization of Sulawesi via Taiwan and the Philippine archipelago.

Von Koenigswald (1935, 1939) proposed that mammals migrated during the Pleistocene era to Indonesia through the Philippines; his hypothesis was based on affinities between animals found in China and Java. An alternative theory postulates that the most probable colonization route for mammals to Sulawesi was through

ancient corridors or land-bridges via Myanmar, Thailand, or Indochina (Bird et al. 2005; De Vos et al. 2007). At present, the relationship between body size gradation and insularism in *Bubalus* and its connection to potential colonization routes, as suggested by Tougaard (2001), remains unknown. However, the specific colonization route of the *Anoa* to Sulawesi remains uncertain.

Tracing the biogeographic history of the *Anoa* group and related species is necessary to determine a possible colonization route for *Anoa*. Many studies have addressed this question using phylogenetic analysis, molecular dating, and reconstruction of ancestral geographic ranges. However, most of these studies focus predominantly on the Bovidae family in general such as *Pseudois* (Tan et al., 2017). Tan et al. (2017) effectively demonstrated the utility of Reconstruct Ancestral State in Phylogenies (RASP) in reconstructing the biogeographic history of the *Pseudois* genus within the Bovidae family. By integrating molecular data with advanced biogeographic modeling, the study provided valuable insights into the evolutionary dynamics and historical factors influencing the distribution of these species.

Molecular and particularly mitochondrial DNA data on *Bubalus* is abundant in GenBank. Mitochondrial DNA is inherited maternally and does not undergo recombination, which allows for clearer lineage tracing and a more straightforward interpretation of phylogenetic relationships. This characteristic makes mtDNA particularly useful for reconstructing colonization histories, as it can serve as a “molecular clock” that reflects historical population dynamics and colonization events more directly than nuclear DNA, which is subject to recombination and more complex inheritance patterns (Hassan et al. 2009; Hassanin et al. 2012; Budakva 2022). Statistical analysis of mitochondrial DNA cytochrome b (cyt b) gene and control region (CR) using Reconstruct Ancestral State in Phylogenies (RASP) has solved numerous biogeographic and historical questions (Banguera-Hinestroza et al. 2014; Lauron et al. 2014; Song et al. 2014; Clementz et al. 2014; Mahmoudi et al. 2017; Tan et al. 2017). The use of RASP is a powerful approach for tracing the biogeographic history of species. RASP allows researchers to reconstruct ancestral geographic ranges based on phylogenetic trees derived from molecular data, particularly mitochondrial DNA, which is advantageous due to its relatively high mutation rate and maternal inheritance patterns Song et al. (2016). This method facilitates the identification of historical colonization routes and the ecological factors influencing species distribution. RASP provides a comprehensive framework for understanding the evolutionary processes that have shaped the distribution of the *Anoa* and its relatives, thereby contributing valuable insights into the dynamics of insular dwarfism and the ecological adaptations of these unique mammals. The present study aims to test two hypothesized colonization routes (land-bridge and insular routes) and trace the biogeographic histo-

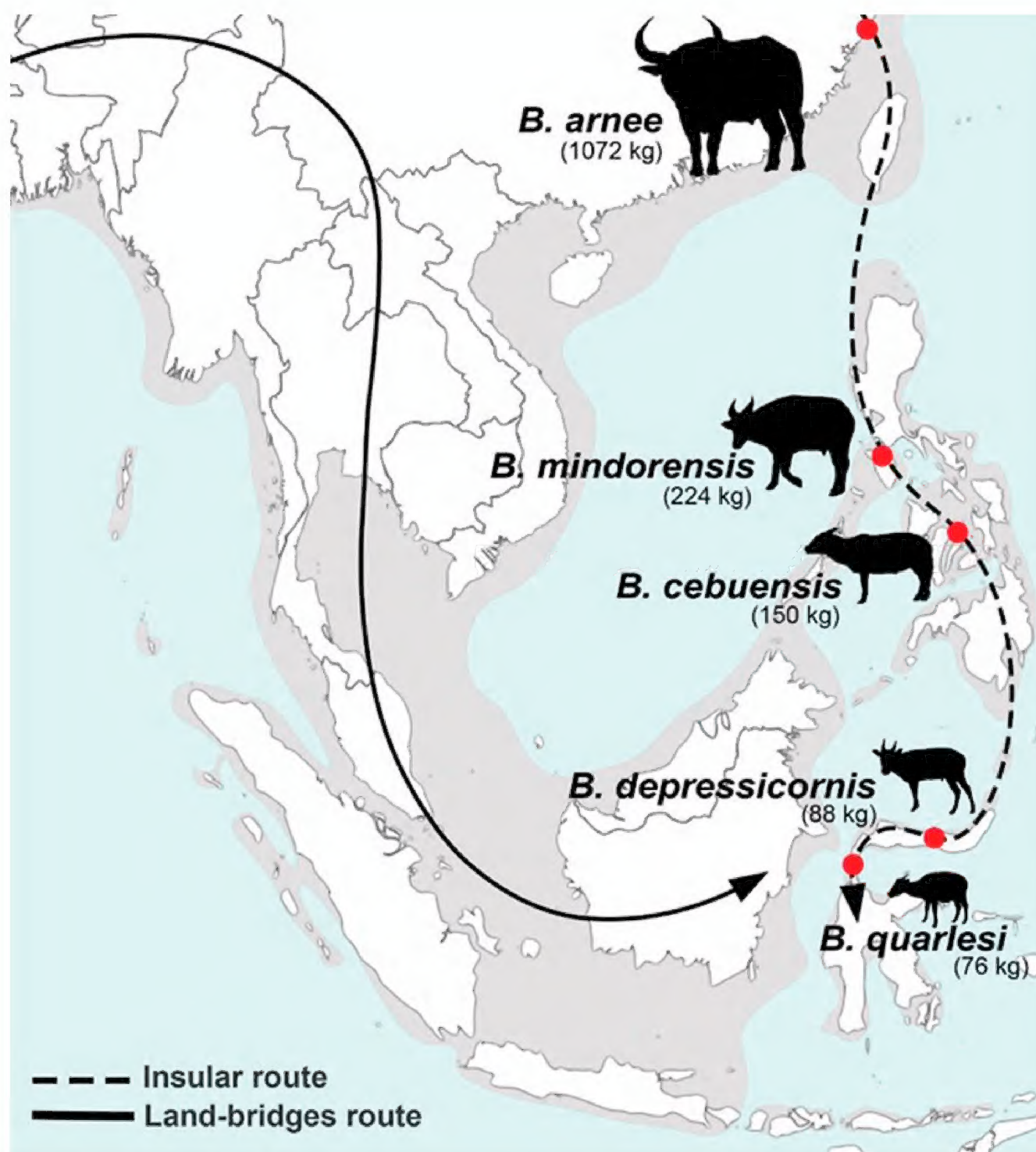


Figure 1. Map of Southeast Asia depicting conditions around the Pleistocene period. The solid line shows the colonization route of large mammals according to the land-bridge hypothesis; dotted lines interpret the colonization route according to the insular hypothesis. This study aims to test these routes using mitochondrial DNA phylogeny to better understand the biogeographic history and evolutionary relationships of the *Anoa* (adapted from Tougaard 2001).

ry of the *Anoa* using mitochondrial DNA phylogeny. This includes examining whether the two *Anoa* species are monophyletic, understanding their evolutionary relationship, and determining the significance of colonization patterns. Investigating these questions provides insight into the evolutionary mechanisms shaping biodiversity on islands and informs conservation strategies for these endangered species.

Materials and methods

Sampling and genomic DNA extraction

A total of 14 *Anoa* fecal samples were obtained in October 2020 from the *Anoa* Breeding Center, Manado, and Bontomarannu Education Park (Suppl. material 1). Eight samples were from lowland *Anoa* (*Bubalus depressicornis*) and six samples were from mountain *Anoa* (*Bubalus quarlesi*). We also collected six water buffalo (*Bubalus bubalis*) fecal samples as a comparison for the genus *Bubalus*. All fecal samples were collected in their

entirety from each animal for genomic DNA extraction. Fecal samples were collected and stored in 50 mL tubes in Longmire's Buffer. Fecal samples were preserved on ice until they arrived in the laboratory for molecular processing. Genomic DNA was extracted from the fecal samples according to the manufacturer's protocol using the QIAamp Fast DNA Stool Mini Kit (Qiagen) and preserved at -25 °C until analysis.

Polymerase chain reaction (PCR) and sequencing

To obtain a fragment of the cyt b gene and CR, we used Primer3 v. 4.0 (bioinfo.ut.ee/primer3-0.4.0) to design a specific primer set based on the lowland *Anoa* sequence (accession number NC020615). Each PCR amplification was performed in a total volume of 25 µL containing 5 µL of 1 × PCR buffer (Promega), 5 µL of 1 × GC enhancer, 1 µL of 0.2 mM dNTPs (Qiagen), 3 µL with 10 pg DNA template, 8.8 µL double-distilled water, 0.2 µL of 0.02 U/µL Taq polymerase (New England, BioLab), and 1 µL with 0.02 µM of each primer. The following primers

were used to amplify cyt b and CR, respectively: CytB_Depress (F), 5'-CATTCATTGACCTCCCTGCT-3'; Cyt B_Depress (R), 5'-GCCGGAACATCATACTTCGT-3'; AFM22, 5'-CGTACGCAATCTTACGATCA-3'; AFM23, 5'-GTAGCTGGACTTAACTGCAT-3'. PCR thermo-cycling conditions were as follows: 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 53.4 °C, 1 min at 72 °C, and a final 10 min at 72 °C. A 1.2% agarose gel was used to visualize the PCR amplicons. The PCR products were then purified and sequenced at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada (UGM). Cyt b and CR sequences were read in both forward and reverse directions to minimize errors and confirm nucleotide integrity, ensuring a reliable consensus sequence for analysis.

Phylogenetic analysis

The sequences were aligned by ClustalW in MEGA 7 (Kumar et al. 2016). Additionally, sequence data for *Anoa* from GenBank were included to supplement the dataset (Suppl. material 1). A phylogenetic tree was constructed according to a Bayesian inference (BI) algorithm using Bayesian evolutionary analysis and tree sampling in BEAST 2.4 (Bouckaert et al. 2014). The best-fit model for this sequence was HKY+G, with $-\ln L = 1984.8411$, ti/tv ratio = 9.6353, $p\text{-invar} = 0$, and Gamma shape = 0.1260, as determined by the Akaike information criterion in jModelTest 2.1.7 (Darriba et al. 2012). BI used Markov Chain Monte Carlo (MCMC) iteration for up to 10,000,000 generations with every tree sample from every 1,000 generations. Out of 10,000 trees produced, 25% of those in the first generation were discarded and designated as “burn-in”. The remaining trees were combined to calculate posterior probability in a 50% majority-rule consensus tree. Branches from BI phylogenetic trees with posterior probability below 0.5 collapsed. The two consensus trees were then edited in FigTree 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Reconstruction of ancestral areas

To determine the biogeographic history of *Anoa* based on the animal's ancestry, we applied a statistical Dispersal Vicariance Analysis (S-DIVA) in a Bayesian framework implemented in RASP 4 (Yu et al. 2015). This method estimates the probability in an ancestral area from every possible phylogenetic node. Using RASP, MCMC Bayesian binary analysis calculated the default uncertainty from phylogenetic inference by optimizing the ancestor's area in multiple trees (Yu et al. 2015). The following geographic regions were analyzed (Fig. 2): Sundaland, which comprises the biogeographic regions of Borneo and Java (representing *Bos javanicus* d'Alton, 1823 and *B. bubalis*); Wallacea, which includes both the lowland regions of Sulawesi, characterized by elevations ranging from 0 to 1000

meters above sea level (representing the lowland *Anoa B. depressicornis*), and highland Sulawesi (representing the mountain *Anoa B. quarlesi*) (Burton et al. 2005); and the Philippines (representing *B. mindorensis*). Bayesian binary MCMC analysis in RASP ran based on a set of Bayesian trees implemented in BEAST (as described above). A dataset including cyt b and CR sequences was used to reconstruct the Bayesian tree with a relaxed-clock model and a prior Yule speciation process tree model. The program was run for 10,000,000 generations and 1,000 generations of tree samples. After 25% of trees were discarded as burn-in, a maximum clade probability was obtained out of the remaining 7,500 trees. Bayesian binary MCMC analysis in RASP was performed on 7,500 post-burn-in trees by applying BEAST maximum clade probability. Analysis was carried out using the evolutionary HKY+G model running for 50,000 cycles with ten chains, sampling every 100 cycles, and discarding 100 trees.

Divergence time estimation

To estimate the time of speciation in *Anoa*, we used a Bayesian relaxed molecular clock model analysis implemented in BEAST and based on a dataset with cyt b mitochondrial DNA. For calibration, we chose three time-constraints:

1. within the genus *Bubalus*, the split between *B. depressicornis* and *B. bubalis* was set at 1.3 ± 0.5 million years ago (Mya) (Ropiquet et al. 2012);
2. within the subgenus *Bubalus*, the split between *B. mindorensis* and *B. bubalis* was set at 1.5 ± 0.2 Mya (Tanaka et al. 1996);
3. the split between *B. javanicus* and the genus *Bubalus* was set at 13.68 ± 1.85 Mya (Hassanin and Ropiquet 2004).

As per the Bayesian tree model recommendation above, analysis was performed using the HKY+G model and a log-normal autocorrelated clock relaxation model. We tested the results using a uniform model on divergence time and a prior Yule speciation process tree combined with the previous three calibration points. All calculations were conducted with 10,000 cycles using ten chains, sampling every 100 cycles, and discarding 100 trees.

Results

Phylogenetic analysis

Cyt b (906 bp) and CR (696 bp) *Anoa* sequences were deposited in GenBank under accession numbers [MK455804–MK455817](#) and [MK499408–MK499433](#), respectively. Additional sequences from the genus *Bubalus* and sequences from the genera *Bos* and *Bubalaphus* were retrieved from GenBank, resulting in a total of 71

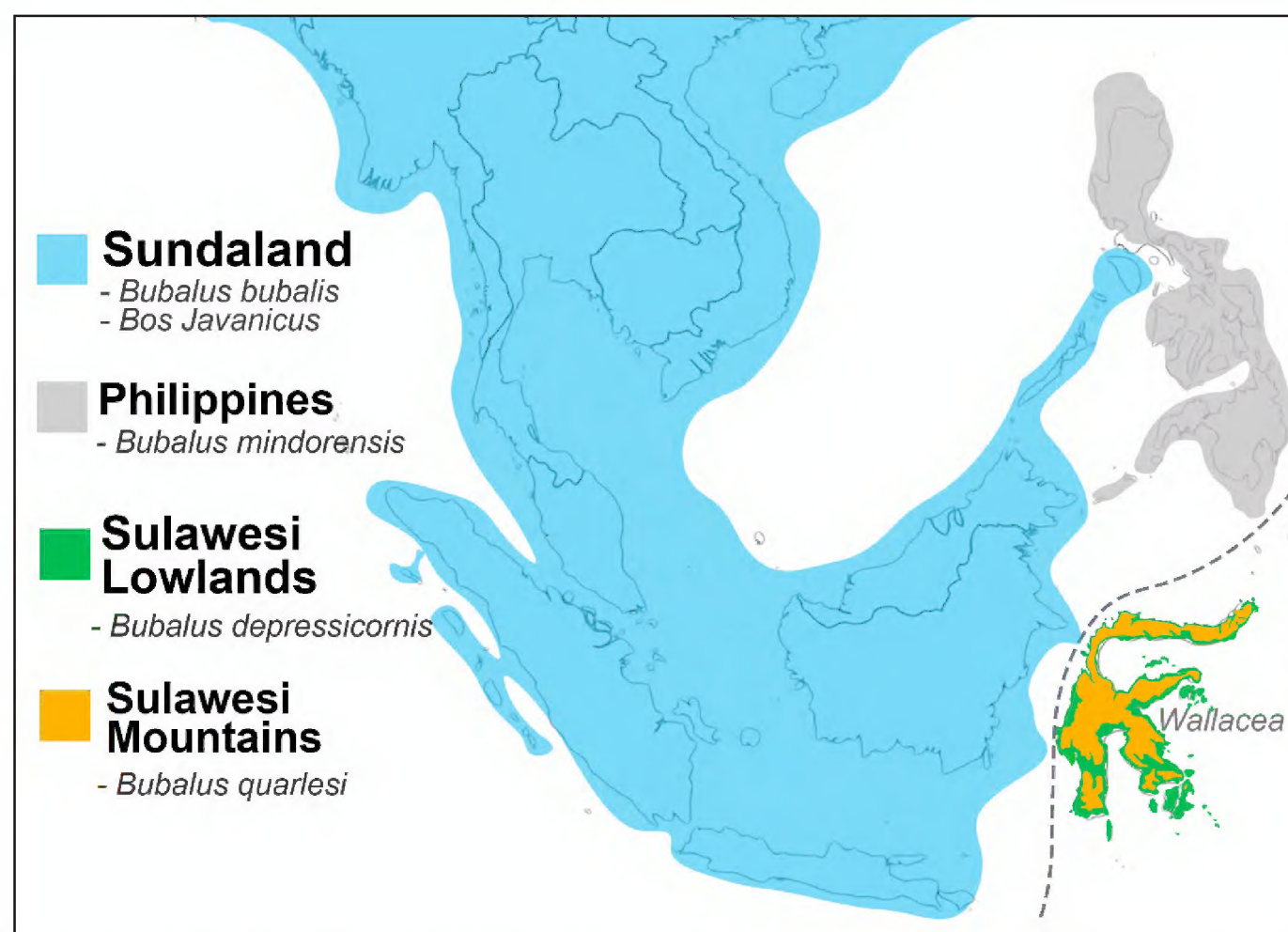


Figure 2. Representative DNA mitochondrial sequence datasets used to reconstruct ancestral areas. Sundaland is represented by *Bos javanicus* and *Bubalus bubalis*, Sulawesi lowland is represented by *Bubalus depressicornis*, Sulawesi mountains are represented by *Bubalus quarlesi*, and the Philippines are represented by *Bubalus mindorensis*.

sequences used for the analysis. The phylogenetic trees constructed in this study include sequences labeled *Bubalus depressions* Charles and *Bubalus depressions* Ferguson. These labels reflect the naming conventions used in the GenBank sequence database and stem from historical inconsistencies in the taxonomy of *Anoa*. Because the BI phylogenetic tree for cyt b and CR showed identical topology, we are showing only the BI cyt b phylogenetic tree (Fig. 3). *Boselaphus tragocamelus* (Pallas, 1766) was used as an outgroup. The phylogenetic tree revealed two main clades: I and II. Clade I comprised all *Anoa* species (mountain and lowland *Anoa*) and included two subclades forming a monophyletic group. Posterior probability of 0.99 and bootstrap value of 100 revealed that lowland and mountain *Anoa* were a sister clade. Clade II comprised the tamaraw (*B. mindorensis*) and the water buffalo (*B. bubalis*), which formed a monophyletic group with high statistical support.

Reconstruction of ancestral areas

MCMC Bayesian binary analysis in RASP using the cyt b gene and CR generated an ancestral area for the various *Bubalus* taxa at genus level (Fig. 4). Every node in the Bayesian binary MCMC tree is represented by a pie chart and assigned a colored portion to indicate the ancestral area's possibility (P) of different taxa on a given tree branch.

Node I represents taxa from the *Bubalus* group. The ancestral area for the genus *Bubalus* comprised the Sundaland area (P, 0.73) for both topologies. Node I then separated into two main groups: Node II (subgenus *Bubalus*) and Node III (subgenus *Anoa*). The subgenus *Bubalus*

included *B. bubalis* and *B. mindorensis*. Sundaland was the most probable ancestral area for Node II (CR P, 0.69; cyt b P, 0.72). Analysis of Node III, which focused on the subgenus *Anoa* (Node III) revealed that *Anoa*'s ancestor inhabited mainly the Sulawesi Mountain area (CR P, 0.42; cyt b P, 0.43) and Sulawesi lowlands (CR P, 0.44; cyt b P, 0.46), with a minor proportion assigned to the Sundaland area (CR P, 0.06; cyt b P, 0.05) but none to the Philippines.

Divergence time estimation

The estimated divergence time for the *Bubalus* group was obtained by a time-calibrated tree formed with BEAST and based on BI topology (Fig. 5). The data show that *Bubalus* diverged during the Early to Middle Pleistocene 1.6 Mya (highest posterior density interval (HPD), 1.36–1.87 Mya). The subgenus *Anoa* diverged during the Middle Pleistocene 1.42 Mya (HPD, 1.14–1.71), whereas the subgenus *Bubalus* diverged during the Middle Pleistocene 1.35 Mya (HPD, 1.07–1.64).

Discussion

Studies on insular dwarfism, such as those by Meiri (2007) and Lomolino (2005), emphasize the significance of ecological factors influencing body size evolution in island environments. RASP's ability to integrate phylogenetic uncertainty into biogeographical reconstructions allows researchers to understand better the dynamics of size evolution in isolated populations (Nylander et al. 2008). This integration is crucial for elucidating the mechanisms

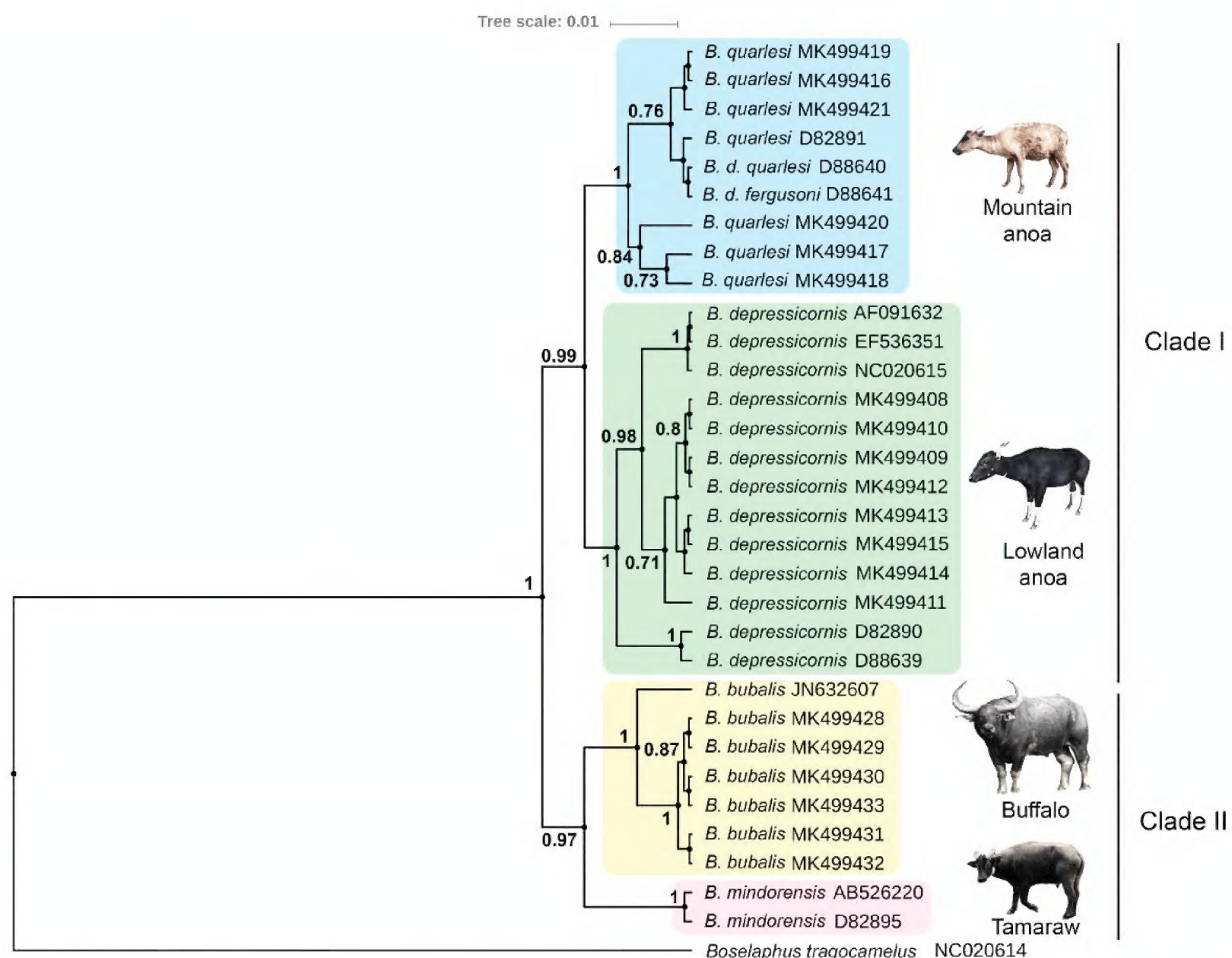


Figure 3. Bayesian phylogenetic tree of mtDNA cyt b gene among *Anoa* and related species, with posterior probabilities ($P > 0.70$) shown on branches. The colors indicate clades: blue for Clade I (mountain *Anoa* *Bubalus quarlesi* and lowland *Anoa* *Bubalus depressicornis*), green for lowland *Anoa*, and yellow for Clade II (buffalo *Bubalus bubalis* and tamaraw *Bubalus mindorensis*). The CR tree exhibited the same topology.

behind insular dwarfism, as demonstrated in analyses of various taxa, including lizards and mammals (Meiri 2007; Geer and Lyras 2016).

This study included *Boselaphus* as an outgroup in phylogenetic tree to clarify the evolutionary relationships within the *Bubalus* genus and its subgenus *Anoa*. Including *Boselaphus* as an outgroup helps better understand the divergence between *Anoa* and other species of the *Bovidae* family. Previous studies, such as those by Pitra et al., (1997), suggested a closer relationship between the lowland *Anoa* and *Boselaphus* using non-coding regions of aromatase cytochrome P450 and lactoferrin. However, these studies were limited by small sample sizes (only 1–2 *Anoa* samples). In contrast, our phylogenetic analysis demonstrates that *Boselaphus* is more distantly related to *Bubalus*, reinforcing the classification of *Anoa* as a subgenus within *Bubalus*, rather than suggesting a closer relationship with *Boselaphus*. Most morphological and genetic studies seem to agree with our findings, grouping *Anoa* into the *Bubalus* genus rather than the *Boselaphus* one (Schreiber et al. 1993; Kakoi et al. 1994; Kikkawa et al. 1997; Burton et al. 2005; Groves and Grubb 2011; Rozzi 2017). Recent genetic studies, including those by subgenus Kakoi et al. (1994) and Tanaka et al. (1996), have suggested that *Anoa* is a subgenus of *Bubalus*.

The phylogenetic tree of *Bubalus*, based on this mitochondrial DNA, revealed two distinct clades: Clade I, which includes *Bubalus arnee* and *Bubalus cebuensis*, and Clade II, which includes *Bubalus bubalis* and *Bubalus mindorensis*. This topology supports the classification of *Anoa* species (*Bubalus quarlesi* and *Bubalus depressicornis*) as part of the *Bubalus* genus, specifically within the subgenus *Anoa*. The separation of *Anoa* species in Clade I from *B. bubalis* and *B. mindorensis* in Clade II highlights the distinct evolutionary trajectory of *Anoa*, suggesting a unique biogeographic history compared to other *Bubalus* species.

The designation of specimens as *B. d. quarlesi* and *B. d. fergusonii* across all phylogenetic trees in this study follows the naming conventions used in the GenBank sequence database, which stem from historical inconsistencies in the nomenclature of *Anoa*. This taxonomic ambiguity, resulting from the morphological similarities and overlapping ecological niches of *Bubalus depressicornis* and *Bubalus Charles*, complicates their classification. The Biological Species Concept, which focuses on reproductive isolation, may not fully apply to these species due to their restricted geographical ranges and potential hybridization in overlapping habitats (Burton et al. 2005; Frantz et al. 2018; Priyono et al. 2018, 2022).

The RASP analysis conducted in this study suggests that the colonization route of *Anoa* in Sulawesi likely

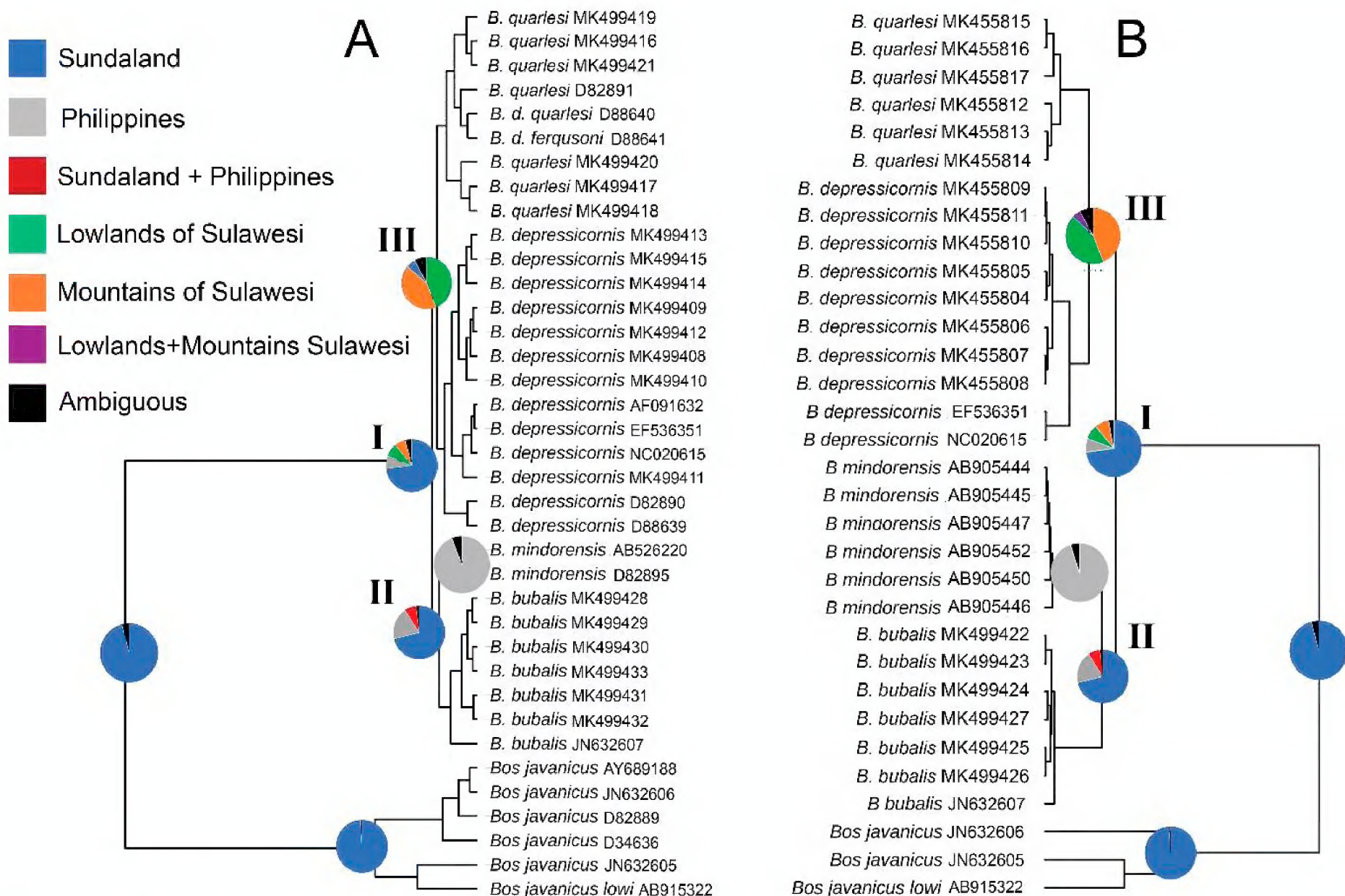


Figure 4. Ancestral area reconstruction in *Anoa* and related species: **A.** cyt b; **B.** CR. Pie charts on each node show the probability of each ancestral node having occurred at an inferred ancestral geographic location using the RASP method. Geographic locations are shown in different colors.

occurred through Sundaland, rather than via the insular route through the Philippines. Previous molecular studies on the *Bubalus* distribution in Indonesia have also suggested that *Bubalus* migrated from mainland Asia to Sundaland and then onward to the western part of Indonesia (Sumatra and Java) (Barker et al. 1997; Lau et al. 1998). These studies are in agreement with the fossil record, whereby the oldest water buffalo and other large herbivores in the Southeast Asia archipelago were found within the Sundaland region during the Pleistocene (Pitra et al. 1997; Price and Webb 2006; Louys et al. 2007; Hassanin et al. 2012). During prehistoric time, dry spells were common and they formed a sort of land corridor that connected mainland Asia with the Indonesian Archipelago (Cockrill and Mahadevan 1974; Nachtsheim and Stengel 1977). Our result suggests that *Bubalus* used a land-bridge corridor from mainland Asia to the Indonesian Archipelago. This result complies with the fossil record, which indicates that the oldest modern water buffalo ancestor, *Bubalus palaeokerabau* (Dubois, 1908), existed in Cisaat, Java, Sundaland, during ancient times (Louys et al. 2007). At that time, the sea level was lower by 40–50 m, which allowed the Sunda Shelf to connect the Malay Peninsula to Java and Borneo; whereas a decrease of the sea level of 120 m extended the dry land mass to include the Philippines and Sulawesi (Voris 2000; Tougaard 2001; Woodruff and Turner 2009). We

believe that *B. mindorensis*, which is endemic to the Philippines, migrated from Sundaland to the Mindoro Island by way of Borneo and Palawan, Philippine Archipelago. The high similarity between mammals in mainland Asia and Palawan indicates that Palawan is an extension of Sundaland (Esselstyn et al. 2010) and supports the colonization of an ancestor of *B. mindorensis* through the same route.

This shows that the *Anoa*'s ancestor evolved as an endemic species after colonizing the Sulawesi Island from Sundaland rather than from the Philippines. A confirmation of the land-bridges colonization from the nearest parts of Sundaland to Sulawesi (especially from the west or east) is also found in modern mammals of Sulawesi, such as the babirusa (Thenius 1970; Van Den Bergh 1999; Bergh et al. 2001; De Vos et al. 2007), macaque (Abegg 2002; Blancher et al. 2008), and tarsier (Merker et al. 2009). Moreover, during the latest glacial phase, the Makassar strait separating South Sulawesi and the eastern coast of Borneo was only some 50 km wide (Bergh et al. 2001), allowing corridors from Sundaland to the southern or western part of Sulawesi. Additional fossil evidence includes a prehistoric painting (Rozzi 2017) of what appears to be *Anoa*, and which was found in Gua Batti, South Sulawesi. In summary, this and previous findings point to the land-bridges hypothesis as the most likely Sundaland-to-Sulawesi colonization route.

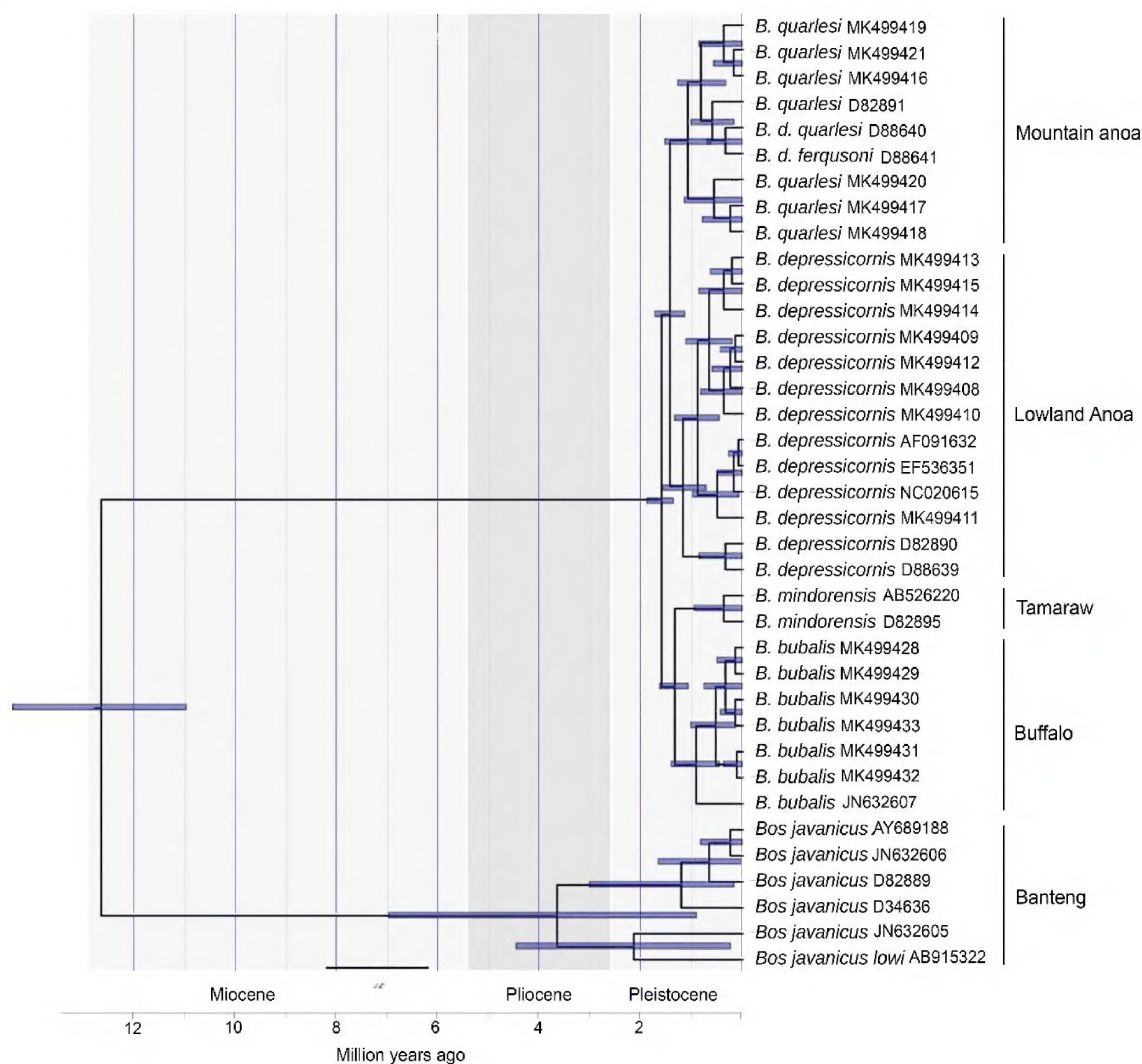


Figure 5. BEAST analysis shows the timeframe estimated for the evolutionary history of *Anoa* and related species in the genus *Bubalus* based on the cyt b gene. Bars at each node indicate the highest posterior density interval.

Previous studies on genomic age estimates have proposed the divergence time for *Bubalus* to be between 2 and 1 Mya (Tanaka et al. 1996; Hassanin and Douzery 1999; Hassanin et al. 2012; Bibi 2013; Bibi and Métais 2016), which implies Early Pleistocene as the time for the first island colonization by mainland water buffalo. This hypothesis agrees with the fossil record's earliest indication of water buffalo and other large herbivores from the Southeast Asia Archipelago on Java during the Pleistocene (Pitra et al. 2004; Louys et al. 2007; Ropiquet et al. 2012). Fossil records indicate as well that the ancestor of the *Anoa* appeared in Sundaland between Early to Middle Pleistocene (Bergh et al. 2001; Tougaard 2001). Other fossil records have revealed that the *Anoa* is closely related to hemibos from Tatrot and the Pinjor formation of the Siwaliks. Magnetostratigraphic analysis assigned the specimens to a time during the Middle-Late Pleistocene (Azzaroli 1983). Calibration of this time interval was dated to between 3.20 Mya and 1.78 Mya

(Spell and McDougall 1992), which is similar to our molecular dating analysis. However, other studies suggest an even longer divergence time for the *Anoa*, for example 12,4 Mya according to Pitra et al. (1997) or 4,3 Mya according to (Stelbrink et al. 2012). The exact time may be influenced by the distant phylogenetic relationship between *Bubalus* species and the close relationship to *Boselaphus* (Pitra et al. 1997) discussed before. In conclusion, our result is supported by previous research which indicates that the *Anoa* is a plesiomorphic member of the Bovini clade and supports the placement of *Anoa* as a subgenus within *Bubalus*, whose ancestor it diverged from around 1.42 Mya. Although our knowledge of the endemic Sulawesi buffalo is hindered by limited sample size, the present study provides important information on the biogeographic history of the *Anoa*, especially its colonization of Sulawesi.

While mitochondrial DNA (mtDNA) analysis provides valuable insights due to its low mutation rate, this meth-

od may limit the resolution of more recent evolutionary changes. Incorporating genomic approaches and expanding the sample size can provide a higher resolution of evolutionary history, enabling more comprehensive genetic diversity and population structure analyses. Integrating genomic data and fossil evidence can refine interpretations of phylogenetic relationships and historical colonizations. In this present study, we tested existing hypotheses for large colonization routes to the Indonesian archipelago using molecular data, with a particular focus on the case of *Anoa*. Our results support the previous hypothesis that the migration of *Anoa* followed one of the major migration routes to the Indonesian archipelago, specifically through Sundaland. In the evolutionary history of *Anoa*, habitat conditions have played a crucial role in preserving their genetic diversity. Mountains and lowland *Anoa* were separated 1.42 million years ago. They are separated due to fragmentation, which is exacerbated by deforestation pressures, leading to a reduction in genetic diversity (Aninta et al. 2024). Connectivity refers specifically to the ability of *Anoa* populations to move between fragmented habitats, maintaining gene flow between isolated populations. Sulawesi is an island with a high level of endemism, so by protecting the two *Anoa* species in Sulawesi, it will also help conserve many other species. Future studies should incorporate fossil records alongside genomic data to build a more complete evolutionary framework for *Anoa*, providing deeper insights into their historical biogeography.

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References

Abegg C (2002) Macaque evolution and dispersal in insular south-east Asia. *Biological Journal of the Linnean Society* 75: 555–576. <https://doi.org/10.1046/j.1095-8312.2002.00045.x>

Aninta SG, Drinkwater R, Carmagnini A, Deere NJ, Priyono DS, Andayani N, Winarni NL, Supriatna J, Fumagalli M, Larson G, Galbusera PHA, Macdonald A, Greer D, Mohamad K, Prasetyaningtyas WE, Mustari AH, Williams JL, Barnett R, Shaw D, Semiadi G, Burton J, Seaman D, Voigt M, Struebig M, Brace S, Rossiter S, Frantz L (2024) The importance of small island populations for the long term survival of endangered large-bodied insular mammals. *bioRxiv*: 595221. <https://doi.org/10.1101/2024.05.23.595221>

Azzaroli A (1983) Quaternary mammals and the “end-Villafranchian” dispersal event – a turning point in the history of Eurasia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 44: 117–139. [https://doi.org/10.1016/0031-0182\(83\)90008-1](https://doi.org/10.1016/0031-0182(83)90008-1)

Banguera-Hinestroza E, Hayano A, Crespo E, Hoelzel AR (2014) Delphinid systematics and biogeography with a focus on the current genus *Lagenorhynchus*: Multiple pathways for antitropical and trans-oceanic radiation. *Molecular Phylogenetics and Evolution* 80: 217–230. <https://doi.org/10.1016/j.ympev.2014.08.005>

Barker JSF, Moore SS, Hetzel DJS, Evans D, Tan SG, Byrne K (1997) Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein-coding loci. *Animal Genetics* 28(2): 103–115. <https://doi.org/10.1111/j.1365-2052.1997.00085.x>

Benítez-López A, Santini L, Gallego-Zamorano J, Milá B, Walkden P, Huijbregts MA, Tobias JA (2021) The island rule explains consistent patterns of body size evolution in terrestrial vertebrates. *Nature Ecology & Evolution* 5: 768–786. <https://doi.org/10.1038/s41559-021-01426-y>

Bergh GD Van Den, Vos J De, Sondaar PY (2001) The Late Quaternary palaeogeography of mammal evolution in the Indonesian Archipelago. *Palaeogeography, Palaeoclimatology, Palaeoecology* 171(3/4): 385–408. [https://doi.org/10.1016/S0031-0182\(01\)00255-3](https://doi.org/10.1016/S0031-0182(01)00255-3)

Bibi F (2013) A multi-calibrated mitochondrial phylogeny of extant Bovidae (Artiodactyla, Ruminantia) and the importance of the fossil record to systematics. *BMC Ecology and Evolution* 13: 166. <https://doi.org/10.1186/1471-2148-13-166>

Bibi F, Vrba ES (2010) Unraveling Bovine Phylogeny: Accomplishments and Challenges. *BMC Biology* 8: 50. <https://doi.org/10.1186/1741-7007-8-50>

Bibi F, Métais G (2016) Evolutionary History of the Large Herbivores of South and Southeast Asia (Indomalayan Realm). In: Ahrestani F, Sankaran M (Eds) *The Ecology of Large Herbivores in South and Southeast Asia*. Ecological Studies, vol 225. Springer, Dordrecht, 15–88. https://doi.org/10.1007/978-94-017-7570-0_2

Bird MI, Taylor D, Hunt C (2005) Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? *Quaternary Science Reviews* 24(20/21): 2228–2242. <https://doi.org/10.1016/j.quascirev.2005.04.004>

Blancher A, Bonhomme M, Crouau-Roy B, Terao K, Kitano T, Saitou N (2008) Mitochondrial DNA sequence phylogeny of 4 populations of the widely distributed cynomolgus macaque (*Macaca fascicularis fascicularis*). *Journal of Heredity* 99: 254–264. <https://doi.org/10.1093/jhered/esn003>

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A software platform for bayesian evolutionary analysis. *PLOS Computational Biology* 10: e1003537. <https://doi.org/10.1371/journal.pcbi.1003537>

Budakva Y (2022) Determination of the genetic structure of pro-maternal pig breeds of Irish selection using mitochondrial DNA markers. *The Animal Biology* 24: 3–8. <https://doi.org/10.15407/animbiol24.02.003>

Burton JA, Hedges S, Mustari AH (2005) The taxonomic status, distribution and conservation of the lowland anoa *Bubalus depressicornis* and mountain anoa *Bubalus quarlesi*. *Mammal Review* 35: 25–50. <https://doi.org/10.1111/j.1365-2907.2005.00048.x>

Clementz MT, Churchill M, Boessenecker RW (2014) Colonization of the Southern Hemisphere by fur seals and sea lions (Carnivora: Ota-

- riidae) revealed by combined evidence phylogenetic and Bayesian biogeographical analysis. *Zoological Journal of the Linnean Society* 172: 200–225. <https://doi.org/10.1111/zoj.12163>
- Cockrill WR, Mahadevan P (1974) The buffaloes of Latin America. *The Husbandry and Health of the Domestic Buffalo*, 676–707.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772. <https://doi.org/10.1038/nmeth.2109>
- De Vos J, van den Hoek Ostende LW, van den Bergh GD (2007) Patterns in insular evolution of mammals: a key to island palaeogeography. *Biogeography, time, and place: distributions, barriers, and islands*. Springer, 315–345. https://doi.org/10.1007/978-1-4020-6374-9_10
- Esselstyn JA, Oliveros CH, Moyle RG, Peterson AT, McGuire JA, Brown RM (2010) Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. *Journal of Biogeography* 37: 2054–2066. <https://doi.org/10.1111/j.1365-2699.2010.02378.x>
- Frantz LAF, Rudzinski A, Nugraha AMS, Evin A, Burton J, Hulme-Beaman A, Linderholm A, Barnett R, Vega R, Irving-Pease EK, Haile J, Allen R, Leus K, Shephard J, Hillyer M, Gillemot S, van den Hurk J, Ogle S, Atofanei C, Thomas MG, Johansson F, Mustari AH, Williams J, Mohamad K, Damayanti CS, Wiryadik ID, Obbles D, Mona S, Day H, Yasin M, Meker S, McGuire JA, Evans BJ, von Rintelen T, Ho SYW, Searle JB, Kitchener AC, Macdonald AA, Shaw DJ, Hall R, Galbusera P, Larson G (2018) Synchronous diversification of sulawesi's iconic artiodactyls driven by recent geological events. *Proceedings of the Royal Society B: Biological Sciences* 285(1876): 20172566. <https://doi.org/10.1098/rspb.2017.2566>
- Geer AAEVD, Lyras GA (2016) The effect of area and isolation on insular dwarf proboscideans. *Journal of Biogeography* 43(8): 1656–1666. <https://doi.org/10.1111/jbi.12743>
- Groves C, Grubb P (2011) *Ungulate Taxonomy*. JHU Press. <https://doi.org/10.56021/9781421400938>
- Hassan AA, El Nahas SM, Kumar S, Godithala PS, Roushdy K (2009) Mitochondrial D-loop nucleotide sequences of Egyptian river buffalo: Variation and phylogeny studies. *Livestock Science* 125: 37–42. <https://doi.org/10.1016/j.livsci.2009.03.001>
- Hassanin A, Delsuc F, Ropiquet A, Hammer C, van Vuuren BJ, Matthee C, Ruiz-Garcia M, Catzeflis F, Areskoug V, Nguyen TT (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *Comptes Rendus Biologies* 335: 32–50. <https://doi.org/10.1016/j.crv.2011.11.002>
- Hassanin A, Douzery EJP (1999) The Tribal Radiation of the Family Bovidae (Artiodactyla) and the Evolution of the Mitochondrial Cytochrome b Gene. *Molecular Phylogenetics and Evolution* 13(2): 227–243. <https://doi.org/10.1006/mpev.1999.0619>
- Hassanin A, Ropiquet A (2004) Molecular phylogeny of the tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, *Bos sauveli* Urbain 1937. *Molecular Phylogenetics and Evolution* 33(3): 896–907. <https://doi.org/10.1016/j.ympev.2004.08.009>
- Hooijer DA (1949) Mammalian evolution in the Quaternary of southern and eastern Asia. *Evolution* 3: 125–128. <https://doi.org/10.2307/2405546>
- Kakoi H, Namikawa T, Takenaka O, Takenaka A, Amano T, Martojo H (1994) Divergence between the *Anoas* of sulawesi and the asiatic water buffaloes, inferred from their complete amino acid sequences of hemoglobin beta chains. *Zeitschrift fur Zoologische Systematik und Evolutionsforschung* 32: 1–10. <https://doi.org/10.1111/j.1439-0469.1994.tb00466.x>
- Kikkawa Y, Yonekawa H, Suzuki H, Amano T (1997) Analysis of genetic diversity of domestic water buffaloes and *Anoas* based on variations in the mitochondrial gene for cytochrome b. *Animal Genetics* 28(3): 195–201. <https://doi.org/10.1111/j.1365-2052.1997.00101.x>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lau CH, Drinkwater RD, Yusoff K, Tan SG, Hetzel DJS, Barker JSF (1998) Genetic diversity of Asian water buffalo (*Bubalus bubalis*): mitochondrial DNA D-loop and cytochrome b sequence variation. *Animal Genetics* 29(4): 253–264. <https://doi.org/10.1046/j.1365-2052.1998.00309.x>
- Lauron EJ, Loiseau C, Bowie RCK, Spicer GS, Smith TB, Melo M, Sehgal RNM (2014) Coevolutionary patterns and diversification of avian malaria parasites in African sunbirds (Family Nectariniidae). *Parasitology* 142(5): 635–647. <https://doi.org/10.1017/S0031182014001681>
- Lomolino MV (2005) Body size evolution in insular vertebrates: Generality of the island rule. *Journal of Biogeography* 32: 1683–1699. <https://doi.org/10.1111/j.1365-2699.2005.01314.x>
- Louys J, Curnoe D, Tong H (2007) Characteristics of Pleistocene megafauna extinctions in Southeast Asia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 243(1/2): 152–173. <https://doi.org/10.1016/j.palaeo.2006.07.011>
- Mahmoudi A, Darvish J, Aliabadian M, Moghaddam FY, Kryštufek B (2017) New insight into the cradle of the grey voles (subgenus *Microtus*) inferred from mitochondrial cytochrome b sequences. *Mammalia* 81: 583–593. <https://doi.org/10.1515/mammalia-2016-0001>
- Meiri S (2007) Size Evolution in island lizards. *Global Ecology and Biogeography* 16: 702–708. <https://doi.org/10.1111/j.1466-8238.2007.00327.x>
- Merker S, Driller C, Perwitasari-Farajallah D, Pamungkas J, Zischler H (2009) Elucidating geological and biological processes underlying the diversification of Sulawesi tarsiers. *Proceedings of the National Academy of Sciences of the United States of America* 106: 8459–8464. <https://doi.org/10.1073/pnas.0900319106>
- Nachtsheim H, Stengel H (1977) *Vom Wildtier zum Haustier: 10 Tabellen*. Parey.
- Nylander JAA, Olsson U, Alström P, Sanmartín I (2008) Accounting for phylogenetic uncertainty in biogeography: A bayesian approach to dispersal-vicariance analysis of the thrushes (Aves: *Turdus*). *Systematic Biology* 57(2): 257–268. <https://doi.org/10.1080/10635150802044003>
- Pitra C, Fickel J, Meijaard E, Groves C (2004) Evolution and phylogeny of old world deer. *Molecular Phylogenetics and Evolution* 33: 880–895. <https://doi.org/10.1016/j.ympev.2004.07.013>
- Pitra C, Furbass R, Seyfert HM (1997) Molecular phylogeny of the tribe Bovini (Mammalia: Artiodactyla): Alternative placement of the *Anoa*. *Journal of Evolutionary Biology* 10: 589–600. <https://doi.org/10.1046/j.1420-9101.1997.10040589.x>
- Price GJ, Webb GE (2006) Late Pleistocene sedimentology, taphonomy and megafauna extinction on the Darling Downs, southeastern Queensland. *Australian Journal of Earth Sciences* 53: 947–970. <https://doi.org/10.1080/08120090600880842>
- Priyono DS, Solihin DD, Farajallah A, Irawati D, Arini DWI (2018) *Anoa*, dwarf buffalo from Sulawesi, Indonesia: Identification based

- on DNA barcode. *Biodiversitas Journal of Biological Diversity* 19(6): 1985–1992. <https://doi.org/10.13057/biodiv/d190602>
- Priyono DS, Solihin DD, Farajallah A, Purwantara B (2020) The first complete mitochondrial genome sequence of the endangered mountain *Anoa* (*Bubalus quarlesi*) (Artiodactyla: Bovidae) and phylogenetic analysis. *Journal of Asia-Pacific Biodiversity* 13: 123–133. <https://doi.org/10.1016/j.japb.2020.01.006>
- Priyono DS, Solihin DD, Farajallah A, Purwantara B (2022) Genetic diversity of the endangered endemic *Anoa* (*Bubalus* spp): Implication for conservation. *HAYATI Journal of Biosciences* 29: 586–596. <https://doi.org/10.4308/hjb.29.5.586-596>
- Ropiquet A, Hammer C, Hassanin A, Vuuren BJV, Matthee C, Ruiz-garcia M (2012) Comptes Rendus Biologies Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes *Comptes Rendus Biologies* 335(1): 32–50. <https://doi.org/10.1016/j.crv.2011.11.002>
- Rozzi R (2017) A new extinct dwarfed buffalo from Sulawesi and the evolution of the subgenus *Anoa*: An interdisciplinary perspective. *Quaternary Science Reviews* 157: 188–205. <https://doi.org/10.1016/j.quascirev.2016.12.011>
- Rozzi R (2018) Space–time Patterns of Body Size Variation in Island Bovids: The Key Role of Predatory Release. *Journal of Biogeography* 45: 1196–1207. <https://doi.org/10.1111/jbi.13197>
- Schreiber A, Nötzold G, Held M (1993) Molecular and chromosomal evolution in *Anoas* (Bovidae: *Bubalus* spec.). *Journal of Zoological Systematics and Evolutionary Research* 31: 64–79. <https://doi.org/10.1111/j.1439-0469.1993.tb00179.x>
- Shrestha TM, Shrestha S, Koirala S, Parajuli A, Paudyal P, Mishra BK, Sapkota M, Ghimire LN (2019) *Bubalus arnee* Forage under Threat: an assessment of Koshi Tappu Wildlife Reserve, Nepal. *International Journal of Research Studies in Zoology* 4(3): 22–27. <https://doi.org/10.20431/2454-941X.0403004>
- Song Y, Lan Z, Kohn MH (2014) Mitochondrial DNA Phylogeography of the Norway Rat. *PLoS ONE* 9(2): e88425. <https://doi.org/10.1371/journal.pone.0088425>
- Stelbrink B, Albrecht C, Hall R, Rintelen T (2012) The biogeography of Sulawesi Revisited: Is there evidence for a vicariant origin of taxa on Wallace’s “Anomalous Island”? *Evolution* 66(7): 2252–2271. <https://doi.org/10.1111/j.1558-5646.2012.01588.x>
- Tan S, Wang Z, Jiang L, Peng R, Zhang T, Peng Q, Zou F (2017) Molecular phylogeny and phylogeography of genus *Pseudois* (Bovidae, Cetartiodactyla): New insights into the contrasting phylogeographic structure. *Ecology and Evolution* 7(17): 7047–7057. <https://doi.org/10.1002/ece3.3269>
- Tanaka K, Solis CD, Masangkay JS, Maeda K, Kawamoto Y, Nami-kawa T (1996) Phylogenetic relationship among all living species of the genus *Bubalus* based on DNA sequences of the cytochrome b gene. *Biochemical genetics* 34: 443–452. <https://doi.org/10.1007/BF00570125>
- Thenius von E (1970) Zur evolution und verbreitungsgeschichte der Suidae (Artiodactyla, Mammalia). *Zeitschrift für Säugetierkunde* 35: 321–342.
- Tougaard C (2001) Biogeography and migration routes of large mammal faunas in South ± East Asia during the Late Middle Pleistocene: focus on the fossil and extant faunas from Thailand. *Palaeogeography, Palaeoclimatology, Palaeoecology* 168(3/4): 337–358. [https://doi.org/10.1016/S0031-0182\(00\)00243-1](https://doi.org/10.1016/S0031-0182(00)00243-1)
- Van Den Bergh GD (1999) The Late Neogene elephantoid-bearing faunas of Indonesia and their palaeozoogeographic implications. *Scripta Geologica* 117: 1–419. <https://doi.org/10.1086/300861>
- Von Koenigswald GHR (1935) Die fossilen Säugetierfaunen Javas. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen* 38: 188–198.
- Von Koenigswald GHR (1939) The relationship between the fossil mammalian faunas of Java and China, with special reference to early man. *Peking Natural History Bulletin* 13: 293–298.
- Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. *Journal of Biogeography* 27: 1153–1167. <https://doi.org/10.1046/j.1365-2699.2000.00489.x>
- Woodruff DS, Turner LM (2009) The Indochinese – Sundaic zoogeographic transition: a description and analysis of terrestrial mammal species distributions. *Journal of Biogeography* 36(5): 803–821. <https://doi.org/10.1111/j.1365-2699.2008.02071.x>
- Yu Y, Harris AJ, Blair C, He X (2015) RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Molecular Phylogenetics and Evolution* 87: 46–49. <https://doi.org/10.1016/j.ympev.2015.03.008>

Supplementary material 1

Information of samples used in this research

Authors: Dwi Sendi Priyono, Dedy Duryadi Solihin, Achmad Farajallah, Bambang Purwantara

Data type: docx

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